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BIOCHEMICAL OXIDATION OF DAIRY WASTES

V. A Review *

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In response to the requests of many members of the dairy industry, the U. S. Department of Agriculture decided in 1947 to initiate research on new and cheaper methods of treating the processing wastes from this important industry. This paper reviews the progress made, with especial attention to the possible application of the results in treating municipal sewage.

The research undertaken was decided upon after consultation with the Task Committee on Dairy Waste Disposal of the Dairy Industry Committee. The research subcommittee of this group, composed of H. A. Trebler of National Dairy Research Laboratory; M. D. Sanders of Swift and Co.; and A. J. Steffen, Chairman, of Wilson and Co., has been especially valuable because of their advice and encouragement. The following six points are quoted from the notes of a meeting on August 12, 1947, when Dr. Trebler came to the laboratory and stated what he thought were the problems to be studied:

"1. Is the 50 per cent reduction in B.O.D. obtained by 24-hr. aeration of milk wastes a chemical or biochemical process?

"2. Can a rapid aerobic fermentation be developed?

"3. Could the Dow aerobic process (used on chemical process wastes) be adapted to milk wastes?

"4. What is the B.O.D. of the various constitutents of milk, and in what order are they oxidized?

"5. A chemical test for B.O.D. would be of great value. This has been tried before, but with limited success.

"6. Development of a non-plugging aeration system would be an immediate contribution."

It is believed that answers to the first five problems are at hand. Considerable additional information has been obtained and a number of studies are under way which could not have been predicted five years ago.

A 10-gal. fermenter or aerator with a mechanical impeller was used for studying the aeration process. The rate of feed, temperature, air flow, and agitation were closely controlled, so that a constant environment would be provided. Analyses of the influent and effluent were made for protein, lactose, and oxygen consumed by dichromate (O.C.). The O.C. test was extremely valuable. Its development and relation to other tests for oxygen demand are discussed later. With these tools, a start was made on answering the questions raised.

Biological Synthesis

A 50 per cent reduction in O.C. was obtained in numerous experiments, confirming previous results. Moreover, analytical tests showed that only one reaction was occurring:

$$\text{Milk solids} + O_2 \rightarrow \text{Bacterial cells} + CO_2 \quad (1)$$

There were essentially no by-products, neither were there unused casein

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and lactose of milk. A biochemical reaction occurs in which the soluble milk constituents are converted to bacterial cells. The yield of cell substance was slightly more than 50 per cent (7). These results answered the first question; the reaction was a specific biochemical transformation of organic nutrients to cell material, or, more plainly, growth of cells.

Rapid Aerobic Fermentation

The development of a rapid aerobic fermentation on the basis of these results was next studied. It was shown that 1,000 p.p.m. of skim milk solids were completely converted to cells in 5 to 6 hr. (4). The rate of this reaction in a mixture containing 500 p.p.m. sludge solids was independent of the amount of milk solids above 100 p.p.m. and fell off markedly only below 50 p.p.m. The rate of oxidation was independent of oxygen tension above 0.5 p.p.m. Also, the rate of oxidation was found to be 0.4 p.p.m. O_2 per minute in one study and as high as 1 p.p.m. O_2 per minute in other experiments (8). Those familiar with the field can appreciate the engineering problems in dispersing air fast enough to supply 1 p.p.m. O_2 per minute.

The separation of the cells from the liquors by centrifuging left an effluent containing only about 10 per cent of the original chemical oxygen demand (7). There is recent evidence that this 10 per cent is primarily bacterial cells, for centrifugation at higher RCF results in a B.O.D. reduction of more than 95 per cent. These results were obtained in the laboratory aerator, which has vigorous mechanical action. The best means of aeration and of separation of the cells or sludge on a practical scale are major phases of the pilot plant work in progress at Pennsylvania State College by contract between the United States Department of Agriculture and the college.

Partial Treatment Process

Another possible way of using this aeration process was studied and recommended (6). It is simply aeration to bring about the conversion of milk solids to cells and the discharge of the aerated mixture without removal of the bacterial cells. Because of the lower rate of oxidation of the cells, or greater relative stability, as it is often called, the reduction in 5-day B.O.D. obtained is about 75 per cent. Such a partial treatment would have two possible applications. First, in many extremely small plants, or in larger ones where complete treatment is not required, it would substantially reduce pollution. Second, the effluent from this partial treatment would be amenable to treatment in a municipal sewage treatment plant. One such arrangement has been installed and is reported to work well. The dairy plant wastes enter the sewage treatment plant separately and are aerated in one tank, then flow into the second tank, where the municipal sewage enters; both are aerated there. The sludge is returned to the first tank.

It was proposed, also, that the aeration be on a fill-and-draw basis, with continuous aeration during the draw-off period, which would be during the night when domestic sewage flow is low. In this system the partly treated effluent would be discharged to the sewer system, and there would be no need for a separate line from the dairy plant to the treatment plant, an obvious impossibility in many cases.

These proposals are based on laboratory studies and cannot be considered fully developed treatment systems. They go a long way, however, toward answering the second question. The third one, consideration of the Dow process, is also answered in a general way. The essential features of the Dow process are the stimulation of a rapid bacterial action and the sweeping out of volatile organic matter, both of which are accomplished by vigorous aeration. Only a small portion of the pollution in milk wastes is volatile, so the latter feature would be of no importance, but rapid biological oxidation, and even additional mechanical agitation, would certainly be advantageous.

Conversion to Cell Solids

A study of the rate and extent of oxidation of the skim milk solids by "activated sludge" was most enlightening (4)(8). Casein and lactose were found to be oxidized at the same rate. It was found that only 35 to 40 per cent of the oxygen required for the complete oxidation was consumed. The composition of the cells was determined and complete balanced chemical equations for the growth of the bacteria were worked out. Although these data were all obtained on the oxidation of milk solids, there is good reason to believe that the general principles hold for other industrial wastes and even for sewage. Bacteria have a relatively constant composition regardless of the source of nutrients. For example, the composition of the organisms recovered from the aeration of dairy waste (7) can be compared with that of the commercial sludge product prepared at the Milwaukee sewage treatment plant (11). The latter product is contaminated with sand, silt, and the iron added as a coagulant. When the analytical data are corrected for these contaminants, the composition of the Milwaukee product is seen to be about the same as that of the dairy waste organisms (Table I).

Another application of the equations developed is the calculation by Eckenfelder (12) of the theoretical amount of sludge produced by an experimental unit treating cannery wastes. He found the actual yield of sludge to be almost exactly that calculated from the data on dairy wastes. Also, preliminary calculations of sludge from sewage treatment indicate rather good agreement with the values predicted from the equations.

TABLE I.—Composition of Dairy Waste Sludge Compared with that of Milorganite

Constituent	Dairy Waste Sludge	Milorganite[1]
Protein ($N \times 6.25$) (%)	67	65
Carbohydrate (%)	14	12
Fat (%)	—	11
Ash (%)	9	11

[1] Corrected for sand and silt (30.2 per cent), Fe_2O_3 (6.1 per cent), and water (6.2 per cent).

Endogenous Respiration

The idea that the primary biochemical reaction is assimilation or growth of cells has a corollary—the further oxidation of these cells by their own metabolism. In studies of bacterial metabolism, this is called endogenous respiration. The question naturally arises whether endogenous processes go on during the rapid growth process. Some years ago strong indirect evidence that it persisted during growth was presented for the symbiotic nitrogen-fixing bacterium, *Rhizobium meliloti* (3). Recently a demonstration that endogenous respiration of a streptomyces persists during growth was made by Cochrane and Gibbs (1). By use of radioactive carbon (C^{14}) they showed that the oxidation of cell carbon goes on unchanged while the cells are growing on unlabeled glucose. It is probable, therefore, that activated sludge maintains its endogenous respiration during growth.

Recently the rate of endogenous respiration for periods up to 48 hr. has been measured (5). The analytical composition of the sludge was found to be $C_5H_7NO_2$. This "formula" cannot be considered to represent any compound; it merely gives the proportion of the oxidizable elements to oxygen. The amount of sludge obtained by the volatile solids determination will have this composition. The endogenous respiration proceeds according to the equation:

$$C_5H_7NO_2 + 5O_2 \rightarrow 5CO_2 + NH_3 + 2H_2O$$
$$113 + 160 \rightarrow 220 + 17 \quad + 36$$

The oxidation of 1 lb. of sludge (volatile solids) requires 160/113 or 1.42 lb. of air. The rate of this oxidation is low; in different experiments it has ranged between 0.5 and 1.0 per cent of the cell tissue oxidized per hour.

Calculation of Solids Balance

From the equations established for the synthesis of cell tissue from milk solids, a yield of 226 units of cells from 424 units of lactose and protein was established, or 53.3 per cent yield of volatile solids. This value is equivalent to the previously reported 58.5 per cent, determined by dry weight, for the sludge containing 8 per cent ash. Thus, a waste containing 1,000 p.p.m. organic matter (not B.O.D.) would produce 533 p.p.m. sludge. The rate of endogenous respiration (or autodigestion) is, at a minium, 0.5 per cent per hour, or 12 per cent per day. If 2,500 p.p.m. sludge (volatile solids) are carried in this system, 300 p.p.m. will be oxidized daily, and sludge will accumulate at a rate of 233 p.p.m. daily. It is obvious that if the rate of endogenous respiration is higher, say 1 per cent per hour, the sludge produced will be completely oxidized and none will accumulate.

These examples are given in detail because a method for determining the rate of oxidation by sludge has recently been developed (10). It is a simple titrimetric measurement of the carbon dioxide produced by the oxidation. Such a determination should be of great use in both industrial waste and sewage treatment.

A number of research and operation control laboratories have requested the details of this method prior to publication. It is hoped that it will be used to measure the oxidative capacity of sludge in enough plants to verify in actual operation the concepts presented here. It would seem that such information would be of great value, both for control of existing plants and for assistance in designing future plants.

Parenthetically, it should be stated that other workers have presented somewhat similar ideas. Heukelekian and co-workers (2) recently developed an equation for the accumulation of sludge solids which has two major portions, a rapid production of sludge and a slow oxidation of it.

The results on the rate and extent of the biochemical reactions occurring in the aerobic oxidation of dairy waste and their possible application to sewage treatment have been discussed in as much detail as space permits, for, as stated in a recent review in THIS JOURNAL, these data and interpretations are probably the laboratory's greatest contribution to this subject to date. It is believed that the results have gone further than anticipated when the original request was made to study the oxidation of milk and its constituents.

Oxygen Consumed *vs.* B.O.D.

Finally, a "chemical test for B.O.D." was requested. This was one of the first problems investigated, for such a test was needed in the experimental work. After study of several alternatives, a simple oxygen consumed (O.C.) method developed by Eldridge was selected, and thousands of determinations have been made in the laboratory. A study was made of the variables in the test, and its applicability to various other wastes was demonstrated (9).

The exact correlation of B.O.D. and O.C. is difficult, if not impossible, for the B.O.D. is a complex of the amount of oxidizable material and its rate of oxidation, whereas O.C. measurements determine the amount of material oxidizable by dichromate under given conditions. For most organic materials, it approximates the 20-day B.O.D., if inhibition, nitrification, and other factors that influence the B.O.D. are ruled out. Cellulose, of course, is rapidly oxidized chemically and is relatively inert biologically.

The correlation between 5-day B.O.D. and O.C. must be attempted, despite the difficulty, for all legal aspects of pollution depend on the B.O.D. test. On theoretical grounds, the ratio B.O.D./O.C. of easily oxidizable milk solids should be higher than 0.68, and this ratio should be lower for the bacterial cells. As discovered previously, the latter are reluctant to oxidize their own tissue. These theoretical considerations have been verified in laboratory tests, but no satisfactory agreement is found in B.O.D. tests on similar samples taken at different times. However, a set of operating results obtained over a two-month period by the National Dairy Research Laboratories was received recently for analysis. Simultaneous analyses of the contents of an aeration tank were made by the two methods. The data are plotted in Figure 1. The line for the lower portion is drawn by allowing equal numbers of points on each side. It has a ratio of B.O.D./O.C. of 0.38, and two-thirds of the values lie within ± 20 per cent of this line.

There are not enough points above 500 p.p.m. O.C. to establish a good line. The dashed line has a slope of 0.8, which would be a reasonable ratio. A line fitted to the 16 points above 500 p.p.m. O.C. would have a slope of slightly greater than 1.0, which would not seem reasonable. It is apparent that more data of this type, obtained in the field, are required before the two methods can be correlated adequately. In the meantime, a value of 0.4 for the ratio of B.O.D./O.C. can be used as a first approximation for bacterial cells or activated sludge.

Volatile Solids of Sludge by O.C. Test

The O.C. method can be used also as a rapid method of determining the organic content or volatile solids of sludge. The empirical factor of 1.25 mg. O_2 per milligram of sludge was established previously for a sludge that had 8 per cent ash (4). If this value is corrected to a volatile solids content,

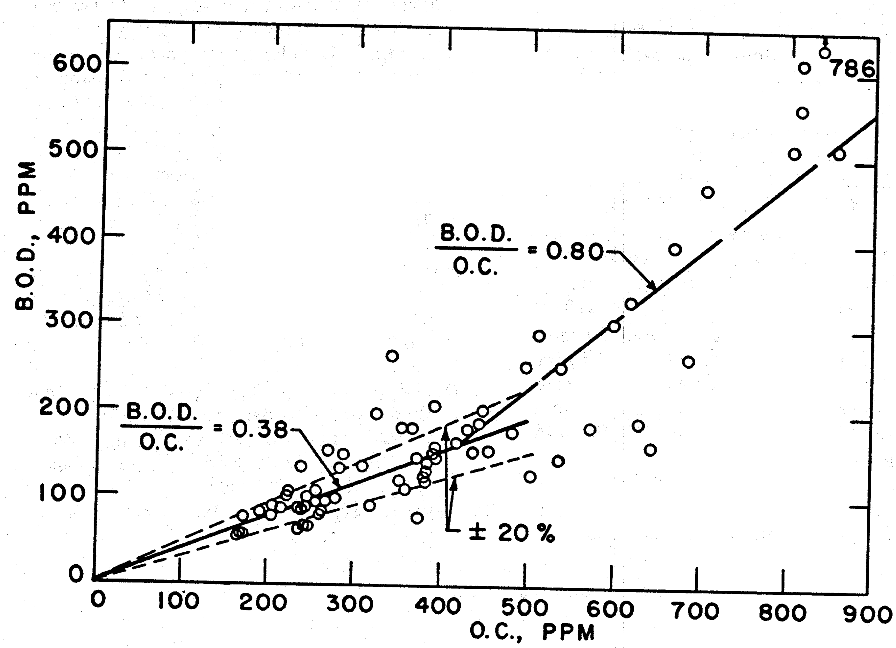

FIGURE 1.—Comparison of B.O.D. with O.C. determinations on contents of aeration tank.

the factor is 1.25/0.92, or 1.36 mg. O_2 per milligram of volatile solids. Eckenfelder (12) has confirmed this value; his determined factor is 1.33 mg. O_2 per milligram of volatile solids.

The sixth of the posed problems has not been studied in this laboratory. It is hoped that successful work on dispersion of air can be done in the pilot-plant studies at Pennsylvania State College. The problem is one for engineering research rather than for biochemical research, and it is believed that it can be investigated more satisfactorily in the pilot plant than in the laboratory. It is a difficult job, which many have attempted to solve.

Summary

Study of the aerobic oxidation of dairy wastes from the biochemical viewpoint has answered many puzzling questions. The essential features are bacterial growth and subsequent oxidation of these bacteria by their own metabolism (endogenous respiration). Therefore, the existing knowledge of bacterial metabolism can be brought to bear on the problem. The relationship between these studies and the oxidation of other industrial wastes and of sewage has been discussed.

Acknowledgment

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References

1. Cochrane, V. W., and Gibbs, M., "The Metabolism of Species of Streptomyces. IV. The Effect of Substrate on the Endogenous Respiration of *Streptomyces coelicolor.*" *Jour. Bact.*, **61**, 305 (1951).
2. Heukelekian, H., Orford, H. E., and Manganelli, R., "Factors Affecting the Quantity of Sludge Production in the Activated Sludge Process." THIS JOURNAL, **23**, 8, 945 (Sept., 1951).
3. Hoover, S. R., and Allison, F. E., "The Growth Metabolism of *Rhizobium*; With Evidence on the Interrelations between Respiration and Synthesis." *Jour. Biol. Chem.*, **134**, 181 (1940).
4. Hoover, S. R., Jasewicz, L., Pepinsky, J. B., and Porges, N., "Assimilation of Dairy Waste by Activated Sludge." THIS JOURNAL, **23**, 2, 167 (Feb., 1951).
5. Hoover, S. R., Jasewicz, L., and Porges, N., "Biochemical Oxidation of Dairy Wastes. IV. Endogenous Respiration and Stability of Aerated Dairy Waste Sludge." THIS JOURNAL, **24**, 9, 1144 (Sept., 1952).
6. Hoover, S. R., Pepinsky, J. B., Jasewicz, L., and Porges, N., "Aeration as a Partial Treatment for Dairy Wastes." Proc. 6th Ind. Waste Conf., p. 313, Purdue Univ. (Nov., 1951).
7. Hoover, S. R., and Porges, N., "Treatment of Dairy Waste by Aeration. II. Continuous Aeration Studies." Proc. 5th Ind. Waste Conf., p. 137, Purdue Univ. (July, 1950).
8. Hoover, S. R., and Porges, N., "Assimilation of Dairy Waste by Activated Sludge. II. The Equation of Synthesis and Rate of Oxygen Utilization." THIS JOURNAL, **24**, 3, 306 (Mar., 1952).
9. Pepinsky, J. B., Porges, N., and Hoover, S. R., "Application of a Rapid Chemical Oxygen Demand Test for Determining Organic Pollutions." Proc. 6th Ind. Waste Conf., p. 367, Purdue Univ. (Nov., 1951).
10. Porges, N., Jasewicz, L., and Hoover, S. R., "Measurement of Carbon Dioxide Evolution from Aerated Sludge." THIS JOURNAL, **24**, 9, 1091 (Sept., 1952).
11. Rehling, C. J., and Truog, E., "Activated Sludge-Milorganite Constituents, Elements and Growth-Producing Substances." *Ind. Eng. Chem.* (Anal. Ed.), **11**, 281 (1939).
12. Eckenfelder, W. W., Jr. Private communications.